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TITLE: Expression of the heterologous genes according to a targeted expression profile

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INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/455; 435/352, 435/463

CLAIMS:

We claim:

1. A method of inserting a gene coding sequence into a target endogenous gene in a eukaryotic cellular host cell genome and expressing said gene coding sequence, by transforming the host cell with a vector comprising a DNA construct, wherein the host cell is a mouse embryonic stem cell and the DNA construct comprises the elements:

5'X-A-P-B-Q-C-Y3'

in which

X and Y are substantially homologous with separate sequences from the target endogenous gene and are of sufficient length to undergo homologous recombination with the host cell genome so as to insert the A-P-B-Q-C elements into the host cell genome;

P is an internal ribosome entry site (IRES);

Q is the gene coding sequence; and

A, B and C are, separately, linker sequences or a covalent bond.

2. The method of claim 1 in which the construct is adapted to insert the gene coding sequence into or in place of the target endogenous gene so that transcription of the gene coding sequence is directed by host regulatory elements for the target endogenous gene.

3. The method of claim 1 in which the construct is adapted to insert the A-P-B-Q-C elements into position 3' (downstream) to the stop codon of an endogenous gene and 5' (upstream) to the polyadenylation signal of the endogenous gene.

4. The method of claim 1 using a construct in which X and Y are each at least 1000 base pairs in length.

5. The method of claim 4 using a construct in which X and Y comprise host elements regulating expression of the target endogenous gene.

6. The method of claim 1 in which the construct additionally comprises a polyadenylation signal at the 3' (downstream) end of the gene coding sequence.

7. The method of claim 1 in which the construct additionally comprises a splice acceptor 5' (upstream) of the IRES.

8. The method of claim 7 in which the splice acceptor is the rabbit .beta.-globin splice acceptor.

9. The method of claim 1 further comprising identifying cells expressing the gene coding sequence.

10. The method of claim 9 in which the gene coding sequence also codes for a selectable marker and the method comprises selecting cells that express the selectable marker.

11. The method of claim 10 in which the selectable marker is a polypeptide that confers antibiotic resistance.

12. A mouse embryonic stem cell comprising an inserted gene coding sequence, wherein the gene coding sequence has been inserted according to the method of claim 1.

13. A descendant of a cell according to claim 12, wherein the descendant has inherited the inserted gene coding sequence.

14. A method of inserting a gene coding sequence into a eukaryotic, cellular host cell genome and expressing said coding sequence under control of elements regulating expression of an endogenous gene in a donor cell genome, said donor cell being a different cell from said host cell, by allowing a DNA construct to undergo random integration into the host cell genome, wherein the host cell is a mouse embryonic stem cell and the DNA construct comprises the sequence:

5'X-A-P-B-Q-C-Y3'

in which

X and Y are homologous with separate sequences from the same donor cell genome and comprise the elements regulating expression of the endogenous gene in the donor cell;

P is an internal ribosome entry site (IRES);

Q is the gene coding sequence; and

A, B and C are, separately, linker sequences or a covalent bond.

15. The method of claim 14 in which the construct additionally comprises a polyadenylation signal at the 3' (downstream) end of the heterologous gene coding sequence.

16. The method of claim 14 in which the construct additionally comprises a splice acceptor 5' (upstream) of the IRES.

17. The method of claim 14 further comprising identifying cells expressing the gene coding sequence.

18. The method of claim 17 in which the gene coding sequence also codes for a selectable marker and the method comprises selecting cells that express the selectable marker.

19. The method of claim 18 in which the selectable marker is a polypeptide that confers antibiotic resistance.

20. A method of inserting a gene coding sequence into a mouse embryonic stem cell genome comprising the steps of:

(i) randomly integrating a DNA construct into a genome using the method of claim 14; followed by

(ii) homologously recombining a DNA construct into the genome using the method of claim 1.

21. A mouse embryonic stem cell comprising an inserted gene coding sequence, wherein the gene coding sequence has been inserted according to the method of claim 14.

22. A descendant of a cell according to claim 21, wherein the descendant has inherited the inserted gene coding sequence.

23. A method for inserting a gene coding sequence into a target endogenous gene in a eukaryotic cellular host cell genome and expressing said gene coding sequence in vitro, by transforming the host cell with a vector comprising a DNA construct, wherein the host cell is an animal stem cell and the DNA construct comprises the element:

5'X-A-P-B-Q-C-Y3'

in which

X and Y are homologous with separate sequences from the same donor cell genome and comprise the elements regulating expression of the endogenous gene in the donor cell;

P is an internal ribosome entry site (IRES);

Q is the gene coding sequence; and

A, B and C are, separately, linker sequences or a covalent bond.

24. A method for inserting a gene coding sequence into a eukaryotic, cellular host cell genome and expressing said coding sequence in vitro under control of elements regulating expression of an endogenous gene in a donor cell genome, said donor cell being a different cell from said host cell, by allowing a DNA construct to undergo random integration into the host cell genome, wherein the host cell is an animal stem cell and the DNA construct comprises the sequence:

5'X-A-P-B-Q-C-Y3'

in which

X and Y are homologous with separate sequences from the same donor cell genome and comprise the elements regulating expression of the endogenous gene in the donor cell;

P is an internal ribosome entry site (IRES);

Q is the gene coding sequence; and

A, B and C are, separately, linker sequences or a covalent bond.

25. A method of inserting a gene coding sequence into a target endogenous gene in a eukaryotic cellular host cell genome and expressing said gene coding sequence, by transforming the host cell with a vector comprising a DNA construct, wherein the host cell is a fertilized non-human egg and the DNA construct comprises the elements:

5'X-A-P-B-Q-C-Y3'

in which

X and Y are substantially homologous with separate sequences from the target endogenous gene and are of sufficient length to undergo homologous recombination with the host cell genome so as to insert the A-P-B-Q-C elements into the host cell genome;

P is an internal ribosome entry site (IRES);

Q is the gene coding sequence; and

A, B and C are, separately, linker sequences or a covalent bond.

26. The method according to claim 23 or 25 in which the construct is adapted to insert the A-P-B-Q-C elements into position 3' (downstream) to the stop codon of an endogenous gene and 5' (upstream) to the polyadenylation signal of the endogenous gene.

27. The method according to any one of claims 23 to 25 in which the gene coding sequence also codes for a selectable marker and the method comprises selecting cells that express the selectable marker.

28. The method according to any one of claims 23 to 25 in which the selectable marker is a polypeptide that confers antibiotic resistance.

29. A method of inserting a gene coding sequence into a eukaryotic, cellular host cell genome and expressing said coding sequence under control of elements regulating expression of an endogenous gene in a donor cell genome, said donor cell being a different cell from said host cell, by allowing a DNA construct to undergo random integration into the host cell genome, wherein the host cell is a fertilized non-human egg and the DNA construct comprises the sequence:

5'X-A-P-B-Q-C-Y3'

in which

X and Y are homologous with separate sequences from the same donor cell genome and comprise the elements regulating expression of the endogenous gene in the donor cell;

P is an internal ribosome entry site (IRES);

Q is the gene coding sequence; and

A, B and C are, separately, linker sequences or a covalent bond.